to the fabric. The stain barrier allows more replicable stain samples to be made, so that stain detection techniques can be accurately compared for the first time.

The presently disclosed methods allow liquid stains to be created on fabric in a reproducible, constant manner so as to limit and hold constant the amount of fabric with which the liquid may interact. In one embodiment, an inert barrier layer is printed onto the fabric to prevent the liquid from interacting with fabric outside the intended area (i.e., the sample area). The stain barrier created using this method insures that each stain spreads within a replicable area of the fabric, thus reducing variability between samples where different dilutions of stain and different fabric substrates are implemented. Now that variation due to sample preparation can be reduced, variation due to dilution, substrate and detection response can be more clearly observed. Thus, more accurate limits of detection can to be determined for stain detection techniques and for the first time, fair comparison of stain detection techniques to one another.

Referring to FIG. **1**, a fabric **10** is shown defining a first surface **12** and an opposite second surface **14**. The fabric can be a woven or nonwoven fabric containing fibers. Any suitable material can be utilized to form the fabric, such as cotton fibers, nylon fibers, polyester fibers, silk fibers, etc.

FIG. **3** shows a 3D printer **30** applying a filament **32** of an inert polymeric composition **20** onto the first surface **12** of the fabric **10**. The 3D printer can be any suitable 3D printer capable of printing a polymeric composition as a filament, and are readily available commercially (e.g., MakerBot® Replicator 2 from MakerBot Industries, LLC in Brooklyn, N.Y.).

The inert polymeric composition **20** generally includes a polymer having a relatively low melting point (e.g., less than 200° C., and particularly less than 150° C.). In particular, the polymer can have a melting point that is between about 100° C. and about 150° C.

Generally, printing of the inert polymeric composition **20** is performed at an extrusion temperature above the glass transition temperature (T_g) of the polymer such that the inert polymeric composition **20** flows into the thickness of the fabric **10** instead of remaining on the surface **12**. For example, in embodiments where the polymer has a glass transition temperature that is between about 50° C. and about 100° C., the inert polymeric composition **20** can be printed at an extrusion temperature of 140° C. to about 150° C. (e.g., about 150° C.). In such an embodiment, the inert polymeric composition **20** can be printed at an extrusion temperature below the melting point of the polymer such that the filament **32** retains some cohesion to inhibit spreading laterally within the fabric **10** or on the surface **12**. However, due to the extrusion temperature being above the glass transition temperature, the inert polymeric composition **20** can penetrate into the thickness of the fabric **10** instead of remaining on the surface **12**, particularly when the fabric is heated before, during, and/or after printing. For example, in embodiments where the polymer has a glass transition temperature that is between about 50° C. and about 100° C., the inert polymeric composition **20** can be printed at an extrusion temperature of 140° C. to about 150° C. (e.g., about 150° C.). In other embodiments, the inert polymeric composition **20** can be printed at an extrusion temperature that is above the melting point of the polymer (no matter the T_g of the polymer) such that the filament **32** melts and flows into the fabric **10** through the surface **12**.

In certain embodiments, the fabric **10** is heated such that the inert polymeric composition **20** completely penetrates its thickness to form the inert polymeric coating **22**. For example, the fabric **10** can be heated prior to printing, during printing, and/or after printing. In one embodiment, the fabric **10** is heated following printing with the inert polymeric composition **20** in an over at a temperature near the melting point of the polymer (e.g., within 15% of the melting point of the polymer) such that the polymer softens and flows through the thickness of the fabric **10**.

The inert polymeric composition **20** in and on the fabric **10** is then cooled to form an inert polymeric coating **22** within and on the fabric **10**, as shown in FIGS. **1** and **2**. Cooling can be accomplished at room temperature (e.g., about 25° C.) up to the melting point of the polymer. In most embodiments, cooling can be achieved by heating the inert polymeric composition up to 100° C.

Generally, the polymer of the inert polymeric composition **20** can be composed of any polymer resin suitable for permeating the fabric **10** during printing while remaining inert to the analyte of the sample. In one embodiment, the polymer resin includes a polylactic acid (PLA) polymer (e.g., PLA having a Tg of about 60° C. to about 65° C. and a melting temperature of about 150° C. to about 180° C.). In one embodiment, a homopolymer of 2-oxepanone (i.e., a polycaprolactone) can be utilized. Polycaprolactone is a biodegradable polyester with a low melting point (e.g., around 60° C.) and a low glass transition temperature (e.g., around −60° C.). Such a polycaprolactone is available commercially under the name LEXIBLE from Perstorp Polyols, Inc., Toledo, Ohio.

The inert polymeric composition **20** can be applied to one or both of the surfaces **12**, **14** of the fabric **10**, depending on the several factors including but not limited to the thickness of the fabric, the viscosity of the inert polymeric composition, the composition of either or both the fabric and the inert polymeric composition, etc. In one particular embodiment, the inert polymeric composition **20** is printed onto both the first surface **12** and the second surface **14**, as well as saturates the thickness of the fabric **10** from the first surface **12** to the second surface **14**.

The inert barrier composition **20** can be applied to the fabric **10** at any amount sufficient to saturate the thickness of the fabric **10**, and upon drying, prevent migration of a liquid sample applied out of the sample area. In particular embodiments, the inert polymeric composition **20** is applied at an add-on weight of about 1% to about 10%, such as about 1% to about 5%.

Once dried and solidified, the inert polymeric composition **20** completely surrounds the protected portion **11** throughout the thickness of the fabric **10** in order to inhibit any substantial flow of a sample through the inert polymeric composition **20** out of the sample area **30**.

Although shown as forming a ring, the inert polymeric composition **20** can for any suitable shape with any suitable size in the fabric **10**.

These and other modifications and variations to the present invention may be practiced by those of ordinary skill in the art, without departing from the spirit and scope of the present invention, which is more particularly set forth in the appended claims. In addition, it should be understood the aspects of the various embodiments may be interchanged both in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention so further described in the appended claims.

What is claimed:

1. A method of forming a sample area on a fabric, the method comprising: